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Analgesic and Anti-Inflammatory Activities of Erythrina Variegata Leaves Extracts

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ABSTRACT

Analgesic and anti-inflammatory activities of *Erythrina variegata* leaves extracts have been tested against various diseases. **Methods:** Water, Ethanol and ethyl acetate extract were prepared from leaves of *E. variegata*. Anti-inflammatory activity and analgesic activity were determined in laboratory model as described by earlier. The anti-inflammatory activity of leaf aqueous extracts showed dose dependent decrease in Carrageenan-induced hind paw edema in Albino rats. The plant extracts also produced dose-dependent analgesic effects against thermally induced nociceptive pain. The above leaves extracts showed higher activity than that of the standard diclofenac. The *E. variegata* was found to possess higher activity at the first and second hour and the activity decreases slowly at third and fourth hours. The present work deals with the determination of Analgesic and Anti-inflammatory studies of *E. variegate*.

Keyword: sensitivity, FNI, morphological parameters, sesame, genetic base

INTRODUCTION

A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal Plants”. Although there no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties. Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In this way indigenous medicinal plants play significant role of an economy of a country. Herbal medicine is relatively cheaper than orthodox medicine, as such it is widely accepted among the people and this could be due to the fact that tradomedical practices blend into the sociocultural life of the people. In recent times, focus on plant research has increased all over the world and large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization [1].

Erythrina has been used in folk medicine for treatment of insomnia, malarial fever, veneral disease, asthma and tooth-ache and as a narcotic and anti-helminthic. The alkaloid Erythroidine was used as a muscle relaxant. Haemoerythrina alkaloids were investigated for their anti-cancer activity [2]. Tanakaa *et al.* has investigated the Eryvarins F and G, two 3-phenoxychromones from the roots of *E. variegate* [3]. Masaru-Sato a has investigated the Synergistic effects of mupirocin and an isoflavanone isolated from *E. variegate* on growth and recovery of methicillin-resistant *Staphylococcus aureus* [4] Jyothirmayi et al. has investigated the Nitrogen extractability and functional properties of defatted *E. variegata* flour [5].

E. variegata belongs to the family *Fabaceae* commonly known as the ‘Indian coral tree’ in Asia or ‘tropical coral’ in the Pacificis an important multipurpose tree species and thrives well in arid and semiarid region. [6]. *E. variegata* is found in many tropical and subtropical regions. An Indian preparation of *E. variegata* is used to destroy pathogenic parasites and relieve joint pain. Juice from the leaves is mixed with honey and ingested to kill tapeworm, Roundworm and threadworm. Women consume the juice to stimulate lactation and menstruation. A warm poultice of the leaves is applied externally to relieve rheumatic joint pains. The bark of *E. variegata* is used as a laxative, diuretic, expectorant, liver ailment and anti- rheumatoid. The leaf extract is used in traditional medicine as nervine sedative, collyrium in opthalmia, anti-asthmatics, anti-epileptic, nematicidal, antiseptic and as an astringent. Leaf paste applied for muscular pain in cattle. The root extract possess antimicrobial activity. [7-10].

It is used medicinally as an antibacterial, anti-inflammatory, antipyretic and antiseptic agent [11]. *Erythrina* has been used in folk medicine for treatment of insomnia, malarial fever, veneral disease, asthma and tooth-ache and as a narcotic and

antihelminthic. The alkaloid Erythroidine was used as a muscle relaxant. Haemoerythrina alkaloids were investigated for their anti-cancer activity [12]. The leaves and tender shoots are eaten as pot-herbs. It was reported that the seeds can be eaten after boiling and roasting. The oil yield reported was 11.3% and the residual seed cake had a protein content of 40%. Phyto-chemical investigation of the non-alkaloidal secondary metabolites of the genus *Erythrina* revealed the presence of one cinnamylphenol some of which exhibit antibacterial and anti-inflammatory activities [13] and inhibit the Na^+/H^+ exchange system. Phyto-chemical analysis of the genus *Erythrina* helped to isolate five isoflavonoids (eryvarins A–E) from the wood and the roots of *E. variega* [14]. *E. variegata* which is rich in crude fat as well as protein. Hence, an attempt has been made to utilise *E. variegata* as a protein source. There is also a report on the description on the effect of extraction parameters, such as pH, ionic strength, solid: solvent ratio and the time of extraction, on the nitrogen extractability and the buffer capacity of *E. variegate* flour [15-17] phytochemical studies on the different parts of dehusked defatted the *E. variegata* plant have led to the isolation of many isoflavones. Isoflavones are compounds present in plant foods, particularly soya beans, which are structurally similar to the mammalian estrogens. They have recently received considerable attention for their potential use in the prevention of postmenopausal bone loss. Data from animal experiments provided evidence that soy protein can attenuate menopausal bone loss, and it was suggested that isoflavones in soya might be responsible for their protective effects on bone [18]. Knowing the above importance, the present work deals with the determination of Analgesic and Anti-inflammatory activities of *E. variegata* leaves.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *E. variegata* were collected from S.F.R College for Women Sivakasi Virudhunagar district Tamil nadu and dried in shade. These were then powdered and stored in air tight container at room temperature until further use. Diclofenac sodium (Standard for both analgesic and anti-inflammatory activity). Wister Albino rats (150-180g) were selected for these studies.

Preparation of Plant Extracts

The coarsely powdered leaf drug of *E. variegata* about 5g was extracted with water, ethanol, ethyl acetate by continuous extraction method using Soxhlet apparatus for 8hrs. The water, ethanol, ethyl acetate extract was concentrated to a dry mass by using water bath. A greenish brown colour residue was obtained. The extracts were undergone to analgesic and anti-inflammatory activities.

Determination of anti-inflammatory activity

Carrageenan induced paw edema method (Fereidoni *et al*., 2000**)**

The inflammatory reaction is readily produced in rats in the form of paw edema with the help of irritants. Carrageenan-induced paw edema is the most commonly used method in experimental pharmacology. Albino rats of Wister strain (80-215 g) were procured from the Animal house. They were housed in standard polypropylene cages and kept under controlled room temperature (25 ± 2^0C) in a 12 hours light-dark cycle. The sex ratio of the experimental animal was 1:1 ratio. The animals were fed on standard laboratory animal diet and food was withdrawn during the experimental hours. All experimental protocols were approved by the animal ethics committee (551/02/C/CPCSEA04/2012-2013/IAEC/CPCSEA).

Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogiston agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility [19].

Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins. Whereas, the second phase is related to the release of prostaglandin. The animals were divided into different groups as Control, Standard, experimental group, each group contain 1-8 animals. Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan in normal Saline, in the right hind paw of the rats, one hour after oral administration of the drugs. The paw diameter was measured with the aid of a vernier caliper at 0, 1, 2, 3 and 4 hours after the injection of carrageenan. The difference between the readings at time zero hour and the different time intervals were taken as the thickness of edema. With Diclofenac sodium (20 mg/kg) as standard, Group 3 were treated orally with water residue of *E. variegata* leaves (200 mg/kg) dose levels of drugs. Group 4 were treated orally with *E. variegate* leaves (400 mg/kg) dose levels of drugs. Group 5 were treated orally with ethanol residue of *E. variegata leaves* (200 mg/kg) dose levels of drugs. Group 6 were treated orally with water residue of *E. variegata* leaves (400 mg/kg) dose levels of drugs. Group 7 were treated orally with Ethyl acetate residue of *E. variegata* leaves (200 mg/kg) dose levels of drugs. Group 8 were treated orally with Ethyl acetate residue of *E. variegata* leaves (400 mg/kg) dose levels of drugs by feeding needle and the paw diameter were measured at 0, 1, 2, 3 and 4 hours after the injection of the standard, *E. variegata* leaves extract and the recorded values were given in Table-1. Percentage inhibition of paw edema is calculated by comparing the controls. The percentage inhibition of inflammation was calculated for each dose at different hours as given below.

Percentage inhibition = 1- Vt / Vc *100
Where Vc = volume of paw edema in control animals
Vt = volume of paw edema in treated animals

Evaluation of Analgesic Activity by Tail Immersion Method (Turner, 1971)

The tail immersion test is carried out as described by standard procedure. The albino rats were selected and last 3.5 cm of their tail was immersed in hot water thermo-statistically maintained at 55^oC, a procedure that caused them to rapidly withdraw their tail [20]. Eight groups of animals were held in position in a suitable restrainer with the tail extending out. The latency to withdraw the tail was recorded with a stopwatch, and a cut-off maximum latency of 15sec was established in order to prevent tissue damage. Group I served as control, which received only vehicle (5mg/kg,i.p). Other groups of animals received one of the following in a similar manner: Diclofenac

(10mg/kg, i.p) and *E. variegata* leaves extract using water, ethanol and ethylacetate as a solvent. The initial reading was taken immediately before administration of test samples and then at 1, 2, 3 and 4 hours after the administration and the recorded data were listed in Table-2

RESULTS

Anti-Inflammatory Studies

Anti-inflammatory activity of water, ethanol and ethylacetate extract of *E. variegata* leaves on Carregeenan- induced paw oedema in rats were observed. The result showed that dose dependent decrease in the value 0.14 to 0.475 for standard diclofenac (20mg/kg) and from 0.215 to 0.115 for extracts (1). Higher activity was observed for 200mg/kg after three hour of incubation which was 96.42% and from 0.0292 to 0.1875 for extract (2). Higher activity was observed for 400 mg/kg for first hour (79.1%) and from 0.18 to 0.1275 for extracts (3). Higher activity was observed for 400 mg/kg for second hour was 80.55%. The above three leaves extracts 400mg/kg of *E. variegata* ethyl acetate extract of *E. variegate* leaves extracts shown higher activity of 80.55% than that of the standard diclofenac. It was presented in the Table 1.

Analgesic Studies

The Analgesic activity of ethanol extract of *E. variegata* leaves have shown the following results. The increase in the basal reaction time from 5.25 to7.5 for standard 10mg/kg diclofenac and 3.5 to 2.5 for *E. variegata* leaves extract were observed. The *E. variegata* was found to possess higher activity at the first and second hour and the activity decreases slowly at third and fourth hour. Among within their compound the analgesic activity decreased at the third and fourth hour when compared to standard. It was presented in the Table 2.

Table.1. Effect of water (1), ethanol (2) and ethylacetate (3) extracts of E. variegata leaves of Anti-inflammatory activity

Group	Dose	Mean (in mm)±SEM			
		1hr	2 hrs	3hrs	4 hrs
Control G1 (Saline)	5ml/kg	0.21±0.04	0.27±0.06	0.36±0.086	0.29±0.089
Standard G2 Diclofenac	20mg/kg	0.14±0.03 (51.78%)	0.09±0.028 (66.97%)	0.7±0.02 (48.21%)	0.48±0.026 (59.66%)
Water G3&G4	200mg/kg p. o	0.22±0.01 (53.57%)	0.17±0.014 (86.11%)	0.14±0.007 (96.42%)	0.12±0.01 (75.78%)
	400mg/kg p. o	0.16±0.02 (8.92%)	0.13±0.017 (36.11%)	0.11±0.015 (50%)	0.12±0.017 (50%)
Ethanol G5 & G 6	200mg/kg p. o	0.09±0.02 (39.28%)	0.07±0.02 (27.77%)	0.06±0.02 (92.14%)	0.033±0.023 (32.57%)
	400mg/kg	0.032±0.001 (79.14%)	0.024±0.002 (73.05%)	0.203±0.036 (71.077%)	0.188±0.0066 (74.66%)
Ethyl Acetate G7 & G 8	200mg/kg p. o	0.133±0.033 (5.35%)	0.14±0.03 (55.55%)	0.123±0.026 (75%)	0.098±0.023 (79.47%)
	400mg/kg p. o	0.18±0.01 (28.57%)	0.163±0.017 (80.55%)	0.143±0.0166 (79.64%)	0.128±0.014 (73.15%)

Table.2. Effect of water (1), ethanol (2) and ethylacetate (3) extracts of *E. variegata* leaves on Analgesic activiy

Group	Dose	Mean time (in seconds) ± SEM			
		1hr	2 hrs	3hrs	4 hrs
Control (Saline) G1	5ml/kg	2.25±0.287	2.25±0.28	2±0.13	2.25±0.32
Standard Diclofenac G2	20mg/kg	5.25±0.37 (57.14%)	5.5±0.33 (59.09%)	6.75±0.28 (70.37%)	7.5±0.33 (70%)

Water G3&G4	200mg/kg p. o	3.75±0.28 (28.57 %)	4±0.47 (27.27%)	5±0.47 (25.92%)	6±0.43 (20%)
	400mg/kg p. o	4.5±0.32 (14.28%)	5.5±0.31 (0%)	6.25±0.55 (7.40%)	7.25±0.28 (3.44%)
Ethanol G5&G6	200mg/kg p. o	3.5±0.41 (33.33%)	5.25±0.32 (4.54%)	3.25±0.27 (51.85%)	2.5±0.29 (66.66%)
	400mg/kg	3.75±0.25 (29.04%)	5.25±0.23 (4.54%)	3.75±0.261 (44.44%)	2.75±0.216 (63.33%)
Ethyl Acetate G7&G8	200mg/kg p. o	2.75±0.275 (47.90%)	3.5±0.25 (36.36%)	4.25±0.31 (37.03%)	2.75±0.19 (63.33%)
	400mg/kg p. o	2.75±0.27 (47.90%)	2.75±0.21 (50%)	3.25±0.267 (51.85%)	3±0.47 (60%)

DISCUSSION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a variety of clinical diseases, though relatively little knowledge about their mode of action is available. *E. variegate* have many medicinal properties, used in folk medicine. In this study, it is clearly shown that it possess both anti-inflammatory activity, Analgesic activity *in vivo* and the activity is based on both time & dose dependent manner. Further research is requiring purifying the phyto-chemical responsible for their activities. Ramiladevi and Manoharan reported that *E. variegate* contains steroids, triterpenoids, flavonoids, furans, sugars, Coumarins, alkaloids, Tannins, phenols and saponins but quinines were absent from the extracts [21]. The species of *Erythrina* possess antiviral, antibacterial, and estrogenic activities [22 & 23]. Furthermore, analgesic and anti-inflammatory effects were observed for an aqueous extract of the stem bark of *Erythrina* sp. [24]. Previously Khare (2007) reported that alkaloids extracted from the leaves of *E. variegata* showed anti-inflammatory and analgesic activity [25]. The present study is also confirmed that *E. variegate* showed analgesic and anti-inflammatory activities even though the plants source is a rain shadow region Sivakasi, Virudhunagar district, Tamilnadu, which receives scanty rainfall with an average of 812mm annually. It was also noted that soil, climate, and other environmental factors might modify the plant bioactive contents [26]. Results from Simpson`s experiments indicate that the biological and chemical profiles of the selected plant species were influenced by the environmental conditions under which the plants were grown [27].

CONCLUSION

E. variegata leaves extract have pharmacological activities. Anti-inflammatory activity of ethanolic extract was found to be appreciable manner. This may be used as standard anti-inflammatory and analgesic drug in the bio market.

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